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### Citation for published version:

Ahmad, H, Requena Navarro, T, Frejo, L, Cobo, M, Gallego-Martinez, A, Martin, F, Lopez-Escamez, JA & Bronstein, AM 2018, 'Clinical and Functional Characterization of a Missense ELF2 Variant in a CANVAS Family', *Frontiers in genetics*. <https://doi.org/10.3389/fgene.2018.00085>

### Digital Object Identifier (DOI):

[10.3389/fgene.2018.00085](https://doi.org/10.3389/fgene.2018.00085)

### Link:

[Link to publication record in Edinburgh Research Explorer](#)

### Document Version:

Peer reviewed version

### Published In:

Frontiers in genetics

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# Clinical and functional characterization of a novel missense *ELF2* variant in a CANVAS family

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**Keywords:** Cerebellar ataxia, Vestibular hypofunction, neuropathy, Whole-exome sequencing, ETS domain.

29

30 **ABSTRACT**

31 Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) is a rare disorder with  
32 **an** unknown etiology. We present a British family with presumed autosomal dominant CANVAS  
33 with incomplete penetrance and variable expressivity. Exome sequencing identified a novel missense  
34 variant in the *ELF2* gene at chr4:g.140058846 C>T, c.10G>A, p.A4T which segregated in all  
35 **affected** patients. By using transduced BE (2)-M17 cells, we found that the mutated ELF2 (mt-ELF2)  
36 gene increased ATXN2 and reduced ELOVL5 gene expression, the causal genes of type 2 and type  
37 38 spinocerebellar ataxias. Both, western blot and confocal microscopy confirmed an increase of  
38 ataxin-2 in BE(2)-M17 cells transduced with lentivirus expressing mt-ELF2 (CEE-mt-*ELF2*), which  
39 was not observed in cells transduced with lentivirus expressing wt-ELF2 (CEE-wt-ELF2). Moreover,  
40 we observed a significant decrease in the number and size of lipid droplets in the CEE-mt-*ELF2*-  
41 transduced BE (2)-M17 cells, but not in the CEE-wt-ELF2-transduced BE (2)-M17. Furthermore,  
42 changes in the expression of ELOVL5 could be related with the reduction of lipid droplets in BE (2)-  
43 M17 cells. This work supports that ELF2 gene regulates the expression of ATXN2 and ELOVL5  
44 genes, and defines new molecular links in the pathophysiology of cerebellar ataxias.

45

## 46 INTRODUCTION

47 The triad of cerebellar ataxia, bilateral vestibulopathy and peripheral neuropathy occurs between 9-  
48 32% of patients with bilateral vestibular failure (Bronstein et al., 1991; Zingler et al., 2007). It is a  
49 rare disorder termed CANVAS (Cerebellar Ataxia, Neuropathy and bilateral Vestibular Areflexia  
50 syndrome; [MIM: 614575]). A review reported 51 patients seen over a 10-year period (Szmulewicz  
51 et al., 2015), in agreement with our own estimates of seeing 6-8 new cases per year.

52 CANVAS is a late-onset, slowly progressive multi-system ataxia likely secondary to a  
53 neurodegenerative ganglionopathy. The combination of cerebellar ataxia and vestibular impairment  
54 produces a characteristic oculomotor sign of impaired (“broken up”) visually enhanced vestibulo-  
55 ocular reflex (Migliaccio et al., 2004). Phenotypic heterogeneity in CANVAS patients is recognized  
56 (Szmulewicz et al., 2014b). Although most cases are sporadic, the finding of 6 affected siblings pairs  
57 (Szmulewicz et al., 2014a) suggests a familial recessive disorder or a dominant inheritance with  
58 incomplete penetrance, **however** the genes involved have not been elucidated.

## 59 Case presentation

60 We describe a non-consanguineous family with 3 CANVAS patients from England (Figure 1A).  
61 Genetic testing **excluded** Friedreich ataxia and SCA 1,2,3,6,7 **and 38 as potential diagnoses**. All  
62 patients **provided** written informed consent **for their participation** for publication and the study  
63 protocol was approved by the institutional review board. Family members in the fourth generation  
64 were examined and remained asymptomatic, **however, symptom onset is typically delayed and**  
65 **usually over 60 years of age**.

66 Patient III:3 (proband), was a **78 year old gentleman** with 20 years of progressive loss of sensation  
67 distally in upper and lower limbs and a gradual deterioration in his balance. He developed oscillopsia  
68 in 2005 **and** in 2014 he noticed **mild slurred** speech and incoordination **followed by development of a**  
69 prominent dry cough, difficulty with micturition and erectile dysfunction. Examination revealed  
70 dysarthria, ataxic gait and a positive Romberg test. Eye movement examination revealed downbeat  
71 nystagmus on lateral gaze. Smooth pursuit was broken horizontally and vertically. Saccades were  
72 moderately hypometric. The doll’s head-eye manoeuvre was abnormally jerky, with numerous  
73 “catch-up” saccades (abnormal visually enhanced vestibulo-ocular reflex, VVOR; (Figure 2).  
74 Horizontal and vertical head impulse tests (HIT) were positive bilaterally. The rest of the cranial  
75 nerve examination was normal. Limb examination revealed normal tone and power throughout with

no spasticity or extrapyramidal features. Reflexes were symmetrically present in the upper limbs however in the lower limbs, ankle jerks were absent and plantars were mute. There was a distal loss to light touch and pinprick sensation in all limbs, vibration sense was absent to the sternum with proprioceptive loss to ankles bilaterally. There was moderate bilateral upper and lower limb dysmetria. **Romberg's test was positive.** Normal blood tests included negative anti-neuronal, anti-GAD, coeliac antibodies, anti treponemal, paraneoplastic antibodies normal B1, B12, glucose, thyroid function, Mg and vitamin E. Bithermal caloric and rotational electronystagmography confirmed bilateral absence of vestibular function. Nerve conduction study (NCS) revealed an axonal sensory neuronopathy. Sural nerve and muscle biopsy were normal. Autonomic function tests were normal. MRI brain showed cerebellar atrophy particularly involving the vermis (Figure 1B). The patient was diagnosed with CANVAS. His father (II:7) died of presumed stroke in his 60's and his mother remained well until she died at the age of 96. Although the clinical record did not report any known neurological condition, II:8 **was considered to be** an obligated carrier. On further exploring the family history, it was discovered that III:6 and III:7 (maternal cousins of proband) had similar symptoms hence were also assessed. Of note, their father (II:11) had a balance disorder of unknown etiology **therefore may have been** affected.

Patient III:6, was **a 78 year old lady** with **a 10 year history** of slowly progressive imbalance, distal numbness and dysesthesiae. Over the last year she described dysphagia and occasional cough. Eye movement examination revealed an abnormal VVOR with head impulse test showing catch up saccades to the left. Pursuit movements were moderately broken up but in keeping with age. There was distal loss to pinprick in upper and lower limbs. Ankle reflexes were absent. She had an ataxic gait and Romberg's was mildly positive. Bithermal caloric testing and rotational test (velocity steps and sinusoidal oscillation), showed significant bilateral reduction of vestibular function. Video-HIT showed consistent abnormal catch up saccades bilaterally. EMG/NCS confirmed a sensory neuronopathy. Autonomic function tests were normal. MRI brain revealed an incidental frontal cavernoma and mild global atrophy. **This was in keeping with a** diagnosis **of** incomplete (*'forme frustre'*) CANVAS phenotype.

Patient III:7, was **a 74 years old lady** with a 2 year history of imbalance, especially in the dark, followed by distal neuropathic symptoms and severe coughing 'fits'. She denied any facial numbness or paresthesiae, speech or swallowing disturbance. Examination revealed a weak downbeat nystagmus in lateral gaze. Pursuit was broken in all directions and saccades were mildly hypometric.

107 She had an abnormal VVOR and bilateral positive HIT. Reflexes were diminished throughout and  
108 ankle jerks were absent. There was distal sensory loss to light touch and pinprick in upper and lower  
109 limbs, proprioceptive impairment to wrists and ankles. Finger-nose testing was mildly impaired in  
110 upper limbs. She had a broad based ataxic gait and Romberg's was positive. Investigations including  
111 cerebellar screening, blood tests and genetic tests were normal. Autonomic function tests were  
112 normal. Bilateral vestibular hypofunction was confirmed on calorics and rotational test. EMG/NCS  
113 confirmed axonal sensory neuropathy with absent sensory nerve action potentials. MRI brain  
114 showed fissural prominence within the superior cerebellar vermis. A cervical spine MRI showed a  
115 slender lower cervical/upper thoracic cord with flattening of the posterior surface and faint signal  
116 change dorsally, compatible with dorsal root ganglionopathy. These features represent a typical  
117 CANVAS phenotype.

118 The fourth subject (III: 2) was a 73 years old lady without any neurological symptoms and a normal  
119 neurological examination.

## 120 **Whole-Exome Sequencing**

121 We sequenced the exomes of 4 individuals in the family (III:3, III:6 and III:7 and III:2) (Figure 1A).  
122 Exons capture, library preparation and sequencing were performed as we previously described, in a  
123 SOLiD 5500xl platform using the reference sequence GRChr37hg19 (Martin-Sierra et al., 2016).  
124 Only variants were considered. Single nucleotide variants (SNV) with coverage >30X and minor  
125 allele frequency (MAF) <0.001 were retrieved using a combined filtering strategy (Requena et al.,  
126 2017). Variants found in the non-affected sibling (III:2) (Figure 1C), were discarded and 3622  
127 variants were retained for further analyses. ANNOVAR software was used to annotate and filter  
128 SNVs. Finally, 30 heterozygous SNVs remained after filtering by exome data from the Exome  
129 Aggregation Consortium, 1000 Genomes databases and in-house controls. Twenty-seven SNVs had  
130 been previously annotated and 3 of them were novel variants. We also used LOD scores derived from  
131 WES-common SNVs to reduce the list of candidate variants, as previously described (Gazal et al.,  
132 2016), and 8 candidate variants remained (Suppl. Table S1). The selected candidate variant, a  
133 missense heterozygous variant in the coding regions of *ELF2* [NM\_201999.2], that segregated with  
134 the phenotype was validated by Sanger sequencing. The candidate variant has been submitted to  
135 ClinVar database (<http://www.ncbi.nlm.nih.gov/clinvar/>).

136 We searched for rare variants in the *ELF2* gene in exome sequencing datasets from two additional  
137 British CANVAS families, and we also performed Sanger sequencing of the *ELF2* gene in these two  
138 families and a third one from Spain. So, a total of 8 additional unrelated individuals with CANVAS  
139 were sequenced, however none of them carried the variant or other rare variants in the coding regions  
140 of *ELF2*.

141 The novel variant leads to a change in the exon 2 of the transcript sequence (p.A4T). The predicted  
142 effect on protein function is probably damaging, since the beginning of the coding sequence is highly  
143 conserved across species and matches with the protein N-terminal elf transcription factor domain,  
144 encoded from 4<sup>th</sup> residue to 108<sup>th</sup> residue (Suppl. Fig. S1 and S2). At protein level, the elf-2 amino  
145 acids sequence has a 67% and 57% of positive homology matches with elf-1 and ets-1, respectively.  
146 The known ETS-binding domain has 87% homology among the 3 transcription factors (TFs), and the  
147 amino acid (p.A4) is conserved in the sequence of *ETS-1*, *ELF-1* and *ELF-2* (Suppl. Fig. S3). A  
148 PAVIVE motif on N-terminal elf transcription factor domain, a relevant recognition motif in elf  
149 family, is conserved between elf-1 and elf-2 amino acids sequences.

#### 150 **BE(2)-M17 cell culture**

151 Human neuroblastoma BE(2)-M17 cell line (ATCC<sup>®</sup> CRL-2267<sup>™</sup>) was cultured and RT-PCR was  
152 used to confirm that the *ELF2*, *ATXN2* and *ELOV5L* genes are constitutively expressed in BE(2)-  
153 M17 neuroblastoma cell line (Suppl Fig. S4 A and C).

#### 154 **Lentiviral vector constructs production and neuroblastoma transduction.**

155 The cDNA encoding for human *ELF2* gene and the *ELF2* gene with the variant described was cloned  
156 in the bicistronic lentiviral vector (LV) pHRSINcppt\_CMVeGFP\_ELF1 $\alpha$ -TetR (also named CEET,  
157 available in our laboratory) using standard molecular biology techniques (PacI/MreI (Sse232I)) to  
158 obtain the lentiviral plasmids CEE-wt-*ELF2* and CEE-mt-*ELF2* respectively. Both LV expressed  
159 eGFP in addition to the wt*ELF2* or the mt*ELF2*. LVs production was performed as previously  
160 described (Frecha et al., 2008). All the LVs used were titrated based on the percentage of eGFP  
161 expressing cells as previously described (Benabdellah et al., 2014).

162 The transduction efficiency was 95%. The number of LV integrated per cell was estimated by qRT-  
163 PCR as previously described (Cobo et al., 2013). Transduction was measured at 3, 7, 10 and 25

164 days). No significant differences were found between both transduced cell lines. Moreover, the  
165 transduction remained stable over time after day 3 (Suppl Fig. S4B).

## 166 **Cell viability and proliferation assays**

167 Cell viability and proliferation assays were performed in BE(2)-M17 cells to investigate the effect of  
168 the *ELF2* variant. For both cell viability and proliferation assays, **there was no difference between the**  
169 **cells.** (Suppl. Fig. S5). These results suggest that overexpression of *wt-ELF2* or *mt-ELF2* gene did  
170 not have any influence on the proliferation or survival of BE(2)-M17 cells and overexpression of  
171 *ATXN2* did not modify the morphology.

## 172 **Functional assays: qRT-PCR, Western blot, immunocytochemistry and confocal microscopy**

173 We **also** investigated the effect of mutant *ELF2* on *ATXN2* and *ELOVL5* expression levels, since  
174 these genes are a direct target of *ELF2*, according to Curated Transcription Factor Targets Dataset  
175 (TRANSFAC), and both have been associated with SCA2 and SCA38 (Scoles et al., 2012; Di  
176 Gregorio et al., 2014; Hoxha et al., 2017).

177 We confirmed that *ELF2*, *ATXN2* and *ELOVL5* genes were constitutively expressed in BE(2)-M17  
178 cells by RT-PCR. **We then** evaluated *ELF2*, *ATXN2* and *ELOVL5* gene expression in CEE-*wt-ELF2*-  
179 and CEE-*mt-ELF2*- transduced BE(2)-M17 cells by qPCR and Western blot and found a significant  
180 increase in both *ELF2* (p=0.03) and *ATXN2* (p=0.002) expression at mRNA levels in the cells  
181 transduced with the CEE-*mt-ELF2*, but not in cells transduced with the CEE-*wt-ELF2* (Figure 3A).  
182 In contrast, *ELOVL5* was significantly decreased (p=0.003) in cells transduced with the CEE-*mt*-  
183 *ELF2*, but not in cells transduced with the CEE-*wt-ELF2* (Figure 3D). The *ATXN2* increase was  
184 confirmed at protein levels in the CEE-*mt-ELF2*- transduced BE(2)-M17 cells, when they were  
185 compared to the wild type cell line (p=0.019, Figure 3A and B).

186 Confocal microscopy imaging illustrated an overexpressed cytoplasmic distribution of ataxin-2 in  
187 CEE-*mt-ELF2*-transduced BE(2)M17 cells. We quantified the fluorescence intensity levels (Fig 3D).  
188 CEE-*mt-ELF2* cell line was the most intensely labelled, followed by those cells that were not  
189 transduced and finally *wt-ELF2* cells. Significant differences were found among non-transduced cells  
190 compared to *mt-ELF2* (p=0.03) and between *wt-ELF2* as compared to *mt-ELF2* (p=0.003, Figure  
191 3C). In addition, the immunocytochemistry showed that the transduction and mutation did not change  
192 *elf2* location.



On comparing non-transduced BE(2)M17 cells with CEE-mt-*ELF2* BE(2)M17-transduced cells, significant differences in the number of lipid droplets were observed with reduced lipid droplets present in the mutant cell line ( $p=0.02$ , Figure 4A and C). In addition, we observed that lipid droplets were smaller in CEE-mt-*ELF2* transduced BE(2)M17 cells ( $0.68\pm0.05$ ) when compared with CEE-*wt-ELF2* BE(2)M17 transduced cells ( $1.53\pm0.14$ ,  $p=1.54\times10^{-8}$ ) and non-transduced BE(2)M17 cells ( $1.83\pm0.03$ ,  $p=1.55\times10^{-48}$ , Figure 4B).

## BACKGROUND

The *ETS* gene family is a group of TFs divided in 12 subfamilies. The *ETS* subfamily includes *ETS1* and *ETS2*; the *ELF* subfamily includes *ELF1*, *ELF2* and *ELF4* (MEF) genes and the *ELG* subfamily consist of *GABPα*. All *ETS* TFs are defined by a highly conserved DNA binding domain, the *ETS* domain with a core GGA(A/T) DNA sequence (Sharrocks, 2001). Previous electromobility shift assays (EMSA) have demonstrated that *ETS1*, *ELF2* and *GABPα* interact with the *ETS* domain within the 5'-UTR in the *ATXN2* gene in HEK293 and SH-SY5Y nuclear lysates. HEK293 cells overexpressing *ETS1* showed an increase in the expression of *ATXN2* gene (Scoles et al., 2012). These findings suggested that the *ETS* domain in *ATXN2* may be regulated by other TFs of the *ETS* gene family such as *ELF-2*. In the present study, we identified a novel missense variant in the *ELF2* gene (E74-like factor 2; *NERF*), which segregates the complete phenotype and we present functional data showing the effect of mutated *ELF2* (mt-*ELF2*) gene on *ATXN2* and *ELOVL5* two genes previously associated with spinocerebellar ataxia 2 and 38 (SCA2 and SCA38). No similar phenotype has been linked to *ELF2* mutations at the time of submission (see Concluding Remarks).

## DISCUSSION

CANVAS is a rare syndrome, with less than 500 cases described worldwide (Szmulewicz et al. 2015), and familial cases have been described rarely (Szmulewicz et al., 2014a). We report a family with 3 CANVAS patients segregating a novel variant in *ELF2* gene. Several lines of evidence support a pathogenic role for the *ELF2* variant in this family. Firstly, multiple bioinformatics tools ranked this variant at the top of the candidate list; secondly, this novel variant was not found in the gnomAD and, perhaps more conclusively, the mt-*ELF2* in a neuroblastoma cell line was able to modify the gene expression of two genes associated with ataxia in two ways. Firstly, by upregulating the expression and translation of *ATXN2* (the gene involved in SCA2) and secondly, by decreasing

the expression and translation of *ELOVL5*, (associated with SCA 38). Sequencing data were re-evaluated in our familial dataset in both genes, but no abnormal CAG repeat expansion in *ATXN2* or pathogenic variants in *ELOVL5* gene such as c.214C>G or c.689G>T were found in the patients.

ELF2 is a TF associated with RUNX1 and both interact in the regulation of gene expression (Wang et al., 1993). We have observed that ELF2 acts as a repressor of *ATXN2* gene expression in neuroblastoma cells and that mt-*ELF2* will not be likely to regulate its expression. Although our mutation is not within the ETS-binding domain, it is not possible to exclude the interaction of ELF2 and other TFs, such as RUNX1.

*ELOVL5* is a target gene for ELF2 according to the TRANSFAC (Wingender et al., 2000; Wingender, 2008) and this gene is considered the causal gene of SCA 38 (Di Gregorio et al., 2014). Our results also confirm that mt-*ELF2* also modifies the expression of *ELOVL5*. This gene is involved in the long-chain fatty acids elongation cycle, and it is highly expressed in Purkinje cells. Furthermore, the *ELOVL5*<sup>-/-</sup> mice develop ataxia and motor impairment during the balance beam test (Hoxha et al., 2017). Several neurological diseases, particularly hereditary spastic paraplegias (Dick et al., 2010; Tesson et al., 2012; Boukhris et al., 2013; Martin et al., 2013) display alterations of lipid metabolism. Increases in lipid droplets play a crucial role in the nervous system and have been associated with *in vitro* models of neurodegenerative disorders such as Huntington's and Parkinson's diseases (Martinez-Vicente et al., 2010; Thiam et al., 2013; Welte, 2015), emphasising the importance of lipid homeostasis in brain membranes.

Although the expression of *ELF2* gene in the human cerebellum is low according to the Allen Brain Atlas (<http://www.brain-map.org/>) (Hawrylycz et al., 2012), and the same variant was not observed in other CANVAS patients, this may be attributed to the genetic heterogeneity commonly found in hereditary ataxias.

Furthermore, we have found strong evidence that the position chr4: g.140058846 C>T in the *ELF2* gene is highly conserved in an evolutionary sense, therefore the variant is likely pathogenic and possibly interferes with protein function. Functional assays indicate a regulatory role of the *ELF2* variant in vitro for two SCA genes, since we have shown that the expression of mt-*ELF2*, but not wt-*ELF2*, increases *ATXN2* gene expression and ataxin-2 translation and decreases *ELOVL5* gene expression in BE(2)-M17 cells.

## CONCLUDING REMARKS

We describe a novel variant in *ELF2* gene in this family with CANVAS syndrome and **demonstrate** its functional effects in *ATXN2* and *ELOV5* genes in BE(2)-M17 transduced cells. The interaction between *ELF2*, *ATXN2* and *ELOVL5* genes found suggests that the regulation of expression in these genes could **potentially** be a shared mechanism in hereditary ataxias.

## **CONFLICT OF INTEREST**

The authors declare that the research was conducted in the absence of any commercial or financial relationships **which** could be construed as a potential conflict of interest.

## **AUTHOR CONTRIBUTIONS**

HA, TR, LF, MC, AG, FM, JALE, and AMB substantially contributed to the conception and design of the work. Patients were examined by both AMB and HA. TR, LF and MC carried out the lab experiments. AG and TR performed bioinformatic analyses of NGS data. All authors analyzed and interpreted the data for the work. All authors drafted the work, revised it critically for important intellectual content and finally approved the version to be published. They all agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## **FUNDING**

This work was supported by an MRC Grant MR/J004685/1 (AMB), financial support to the Otology and Neurotology Group (JALE) and by the Fondo de Investigaciones Sanitarias ISCIII (Spain), Fondo Europeo de Desarrollo Regional (FEDER) from the EU through research grants PI15/02015, TerCel: RD12/0019/0006, and by the CICE and CS de la Junta de Andalucía FEDER/Fondo de Cohesion Europeo (FSE) de Andalucía through research grants PI-57069 (FM) and PI-0407/2012; PI-0318/2014 (MC).

## **ACKNOWLEDGMENTS**

We acknowledge Julia Jaldo and Carmen Martin-Sierra for their assistance with the cell culture. We also thank the assistance of Raquel Marrero Díaz and Sara Moreno Sanjuán of the Microscopy Unit

282 and all the staff of the Genomic Unit at Genyo. We thank Dr Diego Kaski and Mr David Buckwell  
283 with their help for producing (Figure 2). We particularly appreciate the patient's and **their family**  
284 **member's** help and enthusiasm with this study.

285 **SUPPLEMENTARY MATERIAL**

286 The Supplementary Material for this article can be found online

## REFERENCES

- Benabdellah, K., Gutierrez-Guerrero, A., Cobo, M., Munoz, P., and Martin, F. (2014). A chimeric HS4-SAR insulator (IS2) that prevents silencing and enhances expression of lentiviral vectors in pluripotent stem cells. *PLoS One* 9, e84268.
- Boukhris, A., Schule, R., Loureiro, J.L., Lourenco, C.M., Mundwiller, E., Gonzalez, M.A., Charles, P., Gauthier, J., Rekik, I., Acosta Lebrigio, R.F., Gaussen, M., Speziani, F., Ferbert, A., Feki, I., Caballero-Oteyza, A., Dionne-Laporte, A., Amri, M., Noreau, A., Forlani, S., Cruz, V.T., Mochel, F., Coutinho, P., Dion, P., Mhiri, C., Schols, L., Pouget, J., Darios, F., Rouleau, G.A., Marques, W., Jr., Brice, A., Durr, A., Zuchner, S., and Stevanin, G. (2013). Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. *Am J Hum Genet* 93, 118-123.
- Bronstein, A.M., Mossman, S., and Luxon, L.M. (1991). The neck-eye reflex in patients with reduced vestibular and optokinetic function. *Brain* 114 ( Pt 1A), 1-11.
- Cobo, M., Anderson, P., Benabdellah, K., Toscano, M.G., Munoz, P., Garcia-Perez, A., Gutierrez, I., Delgado, M., and Martin, F. (2013). Mesenchymal stem cells expressing vasoactive intestinal peptide ameliorate symptoms in a model of chronic multiple sclerosis. *Cell Transplant* 22, 839-854.
- Di Gregorio, E., Borroni, B., Giorgio, E., Lacerenza, D., Ferrero, M., Lo Buono, N., Ragusa, N., Mancini, C., Gaussen, M., Calcia, A., Mitro, N., Hoxha, E., Mura, I., Coviello, D.A., Moon, Y.A., Tesson, C., Vaula, G., Couarch, P., Orsi, L., Duregon, E., Papotti, M.G., Deleuze, J.F., Imbert, J., Costanzi, C., Padovani, A., Giunti, P., Maillet-Vioud, M., Durr, A., Brice, A., Tempia, F., Funaro, A., Boccone, L., Caruso, D., Stevanin, G., and Brusco, A. (2014). ELOVL5 mutations cause spinocerebellar ataxia 38. *Am J Hum Genet* 95, 209-217.
- Dick, K.J., Eckhardt, M., Paisan-Ruiz, C., Alshehhi, A.A., Proukakis, C., Sibtain, N.A., Maier, H., Sharifi, R., Patton, M.A., Bashir, W., Koul, R., Raeburn, S., Gieselmann, V., Houlden, H., and Crosby, A.H. (2010). Mutation of FA2H underlies a complicated form of hereditary spastic paraplegia (SPG35). *Hum Mutat* 31, E1251-1260.
- Frecha, C., Toscano, M.G., Costa, C., Saez-Lara, M.J., Cosset, F.L., Verhoeyen, E., and Martin, F. (2008). Improved lentiviral vectors for Wiskott-Aldrich syndrome gene therapy mimic endogenous expression profiles throughout haematopoiesis. *Gene Ther* 15, 930-941.
- Gazal, S., Gosset, S., Verdura, E., Bergametti, F., Guey, S., Babron, M.C., and Tournier-Lasserre, E. (2016). Can whole-exome sequencing data be used for linkage analysis? *Eur J Hum Genet* 24, 581-586.
- Hawrylycz, M.J., Lein, E.S., Guillozet-Bongaarts, A.L., Shen, E.H., Ng, L., Miller, J.A., Van De Lagemaat, L.N., Smith, K.A., Ebbert, A., Riley, Z.L., Abajian, C., Beckmann, C.F., Bernard, A., Bertagnolli, D., Boe, A.F., Cartagena, P.M., Chakravarty, M.M., Chapin, M., Chong, J., Dalley, R.A., Daly, B.D., Dang, C., Datta, S., Dee, N., Dolbeare, T.A., Faber, V., Feng, D., Fowler, D.R., Goldy, J., Gregor, B.W., Haradon, Z., Haynor, D.R., Hohmann, J.G., Horvath, S., Howard, R.E., Jeromin, A., Jochim, J.M., Kinnunen, M., Lau, C., Lazarz, E.T., Lee, C., Lemon, T.A., Li, L., Li, Y., Morris, J.A., Overly, C.C., Parker, P.D., Parry, S.E., Reding, M., Royall, J.J., Schulkin, J., Sequeira, P.A., Slaughterbeck, C.R., Smith, S.C., Sodt, A.J., Sunkin, S.M., Swanson, B.E., Vawter, M.P., Williams, D., Wohnoutka, P., Zielke, H.R., Geschwind,

329 D.H., Hof, P.R., Smith, S.M., Koch, C., Grant, S.G., and Jones, A.R. (2012). An anatomically  
330 comprehensive atlas of the adult human brain transcriptome. *Nature* 489, 391-399.

331 Hoxha, E., Gabriele, R.M.C., Balbo, I., Masante, L., Zambelli, V., Mitro, N., Caruso, D., Brusco, A.,  
332 Borroni, B., and Tempia, F. (2017). Elov15 knock-out mice as a model of spinocerebellar  
333 ataxia 38. *Frontiers in Cellular Neuroscience*.

334 Martin-Sierra, C., Requena, T., Frejo, L., Price, S.D., Gallego-Martinez, A., Batuecas-Caletrio, A.,  
335 Santos-Perez, S., Soto-Varela, A., Lysakowski, A., and Lopez-Escamez, J.A. (2016). A novel  
336 missense variant in PRKCB segregates low-frequency hearing loss in an autosomal dominant  
337 family with Meniere's disease. *Hum Mol Genet*.

338 Martin, E., Schule, R., Smets, K., Rastetter, A., Boukhris, A., Loureiro, J.L., Gonzalez, M.A.,  
339 Mundwiler, E., Deconinck, T., Wessner, M., Jornea, L., Oteyza, A.C., Durr, A., Martin, J.J.,  
340 Schols, L., Mhiri, C., Lamari, F., Zuchner, S., De Jonghe, P., Kabashi, E., Brice, A., and  
341 Stevanin, G. (2013). Loss of function of glucocerebrosidase GBA2 is responsible for motor  
342 neuron defects in hereditary spastic paraplegia. *Am J Hum Genet* 92, 238-244.

343 Martinez-Vicente, M., Tallozy, Z., Wong, E., Tang, G., Koga, H., Kaushik, S., De Vries, R., Arias,  
344 E., Harris, S., Sulzer, D., and Cuervo, A.M. (2010). Cargo recognition failure is responsible  
345 for inefficient autophagy in Huntington's disease. *Nat Neurosci* 13, 567-576.

346 Migliaccio, A.A., Halmagyi, G.M., Mcgarvie, L.A., and Cremer, P.D. (2004). Cerebellar ataxia with  
347 bilateral vestibulopathy: description of a syndrome and its characteristic clinical sign. *Brain*  
348 127, 280-293.

349 Requena, T., Gallego-Martinez, A., and Lopez-Escamez, J.A. (2017). A pipeline combining multiple  
350 strategies for prioritizing heterozygous variants for the identification of candidate genes in  
351 exome datasets. *Hum Genomics* 11, 11.

352 Scoles, D.R., Pflieger, L.T., Thai, K.K., Hansen, S.T., Dansithong, W., and Pulst, S.M. (2012). ETS1  
353 regulates the expression of ATXN2. *Hum Mol Genet* 21, 5048-5065.

354 Sharrocks, A.D. (2001). The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol* 2, 827-  
355 837.

356 Szmulewicz, D.J., Mclean, C.A., Macdougall, H.G., Roberts, L., Storey, E., and Halmagyi, G.M.  
357 (2014a). CANVAS an update: clinical presentation, investigation and management. *J Vestib*  
358 *Res* 24, 465-474.

359 Szmulewicz, D.J., Mclean, C.A., Rodriguez, M.L., Chancellor, A.M., Mossman, S., Lamont, D.,  
360 Roberts, L., Storey, E., and Halmagyi, G.M. (2014b). Dorsal root ganglionopathy is  
361 responsible for the sensory impairment in CANVAS. *Neurology* 82, 1410-1415.

362 Szmulewicz, D.J., Seiderer, L., Halmagyi, G.M., Storey, E., and Roberts, L. (2015).  
363 Neurophysiological evidence for generalized sensory neuronopathy in cerebellar ataxia with  
364 neuropathy and bilateral vestibular areflexia syndrome. *Muscle Nerve* 51, 600-603.

365 Tesson, C., Nawara, M., Salih, M.A., Rossignol, R., Zaki, M.S., Al Balwi, M., Schule, R., Mignot,  
366 C., Obre, E., Bouhouche, A., Santorelli, F.M., Durand, C.M., Oteyza, A.C., El-Hachimi,  
367 K.H., Al Drees, A., Bouslam, N., Lamari, F., Elmalik, S.A., Kabiraj, M.M., Seidahmed, M.Z.,  
368 Esteves, T., Gaussen, M., Monin, M.L., Gyapay, G., Lechner, D., Gonzalez, M., Depienne,  
369 C., Mochel, F., Lavie, J., Schols, L., Lacombe, D., Yahyaoui, M., Al Abdulkareem, I.,  
370 Zuchner, S., Yamashita, A., Benomar, A., Goizet, C., Durr, A., Gleeson, J.G., Darios, F.,  
371 Brice, A., and Stevanin, G. (2012). Alteration of fatty-acid-metabolizing enzymes affects

372 mitochondrial form and function in hereditary spastic paraplegia. *Am J Hum Genet* 91, 1051-  
373 1064.

374 Thiam, A.R., Antonny, B., Wang, J., Delacotte, J., Wilfling, F., Walther, T.C., Beck, R., Rothman,  
375 J.E., and Pincet, F. (2013). COPI buds 60-nm lipid droplets from reconstituted water-  
376 phospholipid-triacylglyceride interfaces, suggesting a tension clamp function. *Proc Natl Acad*  
377 *Sci U S A* 110, 13244-13249.

378 Wang, C.Y., Petryniak, B., Thompson, C.B., Kaelin, W.G., and Leiden, J.M. (1993). Regulation of  
379 the Ets-related transcription factor Elf-1 by binding to the retinoblastoma protein. *Science*  
380 260, 1330-1335.

381 Welte, M.A. (2015). Expanding roles for lipid droplets. *Curr Biol* 25, R470-481.

382 Wingender, E. (2008). The TRANSFAC project as an example of framework technology that  
383 supports the analysis of genomic regulation. *Brief Bioinform* 9, 326-332.

384 Wingender, E., Chen, X., Hehl, R., Karas, H., Liebich, I., Matys, V., Meinhardt, T., Pruss, M.,  
385 Reuter, I., and Schacherer, F. (2000). TRANSFAC: an integrated system for gene expression  
386 regulation. *Nucleic Acids Res* 28, 316-319.

387 Zingler, V.C., Cnyrim, C., Jahn, K., Weintz, E., Fernbacher, J., Frenzel, C., Brandt, T., and Strupp,  
388 M. (2007). Causative factors and epidemiology of bilateral vestibulopathy in 255 patients.  
389 *Ann Neurol* 61, 524-532.

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## Figure legends

**FIGURE 1. Genetic diagnosis of familial CANVAS:** (A) Pedigree of an autosomal dominant CANVAS family with three affected cases with the age of onset. (B) Sagittal MRI showing cerebellar atrophy in patient III:3. (C) Chromatogram of reverse chain of the variant chr4: g.140058846 G>A from an affected individual (III.3) is compared to the sequence from a familial control (III.2).

**FIGURE 2. Head and eye horizontal movements in the CANVAS proband.** The patient fixates a visual target on the wall while the examiner manually oscillates his head from behind in a quasi-sinusoidal fashion (visually-assisted vestibulo-ocular reflex or VVOR). The compensatory eye movement elicited is severely broken-up or cog-wheeled due to the presence of multiple eye saccades (best seen as ‘spikes’ in the eye velocity trace). Upwards deflections correspond to rightwards head or eye movements.

**FIGURE 3. *ATXN2* expression in BE(2)M17, wt-*ELF2* and mt-*ELF2* transduced cells.** (A) *ATXN2* qPCR and ataxin-2 Western blot show statistical differences between wt-*ELF2* and mt-*ELF2* transduced cells, both in qPCR and Western blot. (B) Representative western blot of BE(2)M17 exhibiting an increased content of *ATXN2* in mt-*ELF2* transduced cells. *ATXN2* (#611378, 1:1000), *Elf2* (#HPA006057-100UL, 1:1000) GAPDH (#AB2302, 1:3000 and secondary antibodies #HAF007, 1:6000, #HAF008, 1:3000, #A9046-1ML, 1:10000. (C) CTCF emitted by BE(2)M17 cells labelled with anti-ataxin-2 antibody in non-transduced, wt-*ELF2* transduced and mt-*ELF2* cells. (D) Representative immunocytochemistry image of ataxin-2 in non-transduced BE(2)M17, wt-*ELF2* and mt-*ELF2* transduced cells showing an increased staining in mt-*ELF2* cell-line. \*  $p<0.02$ , \*\*  $p<0.002$ . Primary antibodies anti-ataxin-2 (1:250) and anti-*ELF2* (1:500) and visualized with Alexa-555-conjugated goat anti-mouse #A-21422, 1:500 and Alexa-633-conjugated goat anti-rabbit #A-21071, 1:500, respectively. (E) *ELOVL5* qPCR show statistical differences between wt-*ELF2* and mt-*ELF2* transduced cells. \*  $p<0.003$



421 **FIGURE 4. Changes in Lipid droplets in transduced BE(2)M17 cell-lines.** (A) Number of lipid  
422 droplets particles per cell in each cell-line (\* $p=0.02$ ). (B) Mean of particles size in every cell-line.  
423 \*BE(2)M17 non-transduced cells vs wt-*ELF2* cells ( $p=0.03$ ); \*\*wt-*ELF2* vs mt-*ELF2* transduced  
424 cells ( $p=1.54 \times 10^{-8}$ ); \*\*\*BE(2)M17 vs mt-*ELF2* ( $p=1.55 \times 10^{-48}$ ). (C) Representative  
425 immunocytochemistry image of Lipid droplets stained with Nile Red in non-transduced BE(2)M17,  
426 wt-*ELF2* and mt-*ELF2* transduced cells showing a decrease number and size of the droplets in mt-  
427 *ELF2* cell-line. For lipid droplets experiments, cells were stained with Nile red to measure the  
428 number and size of lipid droplets. After Nile red staining, cells were fixed and staining with anti-  
429 *ELF2* (1:500) and visualized with Alexa-633-conjugated goat anti-rabbit (1:500).